EA ROOM H.M. CONFERENCES PAMPHLET ISO

SEPARATUM

Vol. XIV 20-VII-1961 Fasc. 3

MYCOPATHOLOGIA

ET

MYCOLOGIA APPLICATA

FUNDATORES:

R. CIFERRI

P. REDAELLI

EDENDA CURAVIT:

TIBOR BENEDEK

Chicago, Ill.

EDITORES:

S. Akai, Kyoto — F. de Almeida, São Paulo — A. N. Aravysky, Leningrad — A. E. de Arêa Leão, Rio de Janeiro — E. Baldacci, Milano — J. Bánhegyi, Budapest — E. Baudys, Brno — A. L. van Beverwijk, Baarn — F. Blank, Montreal — K. B. Boedijn The Hague — S. R. Bose, Calcutta — Wm. Bridge Cooke, Cincinnati, Ohio — H. Campins, Barquisimeto, Venezuela — A. L. Carrión, San Juan, P. R. — A. Castellani, Lisboa — A. Catanei, Alger — K. Cejp, Praha — A. Chaves Batista, Pernambuco — A. Ciccarone, Bari — N. F. Conant, Durham, N. C. — J. N. Couch, Chapel Hill, N. C. — J. Coudert, Lyon — S. N. Dasgupta, Lucknow — E. D. Delamater, Philadelphia — C. W. Dodge, St. Louis, Mo. — E. Drouhet, Paris — C. W. Emmons, Washington, D.C. R. Evolceanu, Bucharest — E. Fejér, Budapest — G. W. Fischer, Pullman, Wash. — G. Florenzano, Firenze — A. da Fonseca, Rio de Janeiro — N. Fries, Uppsala — E. Gäumann, Zürich — A. Gonzalez Ochoa, Mexico, D. F. — H. Götz, Essen — L. Grigoraki, Marseille — E. I. Grin, Sarajevo — E. Hazen, Albany N. Y. — R. Heim, Paris — S. Ito, Sapporo — D. Janke, Marburg/Lahn — J. Jørstad, Oslo — P. N. Kashkin Leningrad — F. D. Kern, State College, Pa. — A. M. Kligman, Philadelphia, Pa. — H. H. Kuehn, Camden, N. J. — C. da Silva Lacaz, São Paulo — J. Lodder, Delft — J. E. Mackinnon, Montevideo — O. de Magalhaes, Belo Horizonte — F. Mariat, Paris — P. Martens, Louvain — D. S. Martin, Durham, N. C. — J. Meyer, Strasbourg — H. Pereiro Miguens, Santiago de Compostela — J. H. Miller, Athens, Ga. — R. Morquer, Toulouse — E. M. Mrak, Berkeley, Calif. — J. A. Nannfeldt, Upsala — P. Negroni, Buenos Aires — F. L. Niño, Buenos Aires — J. Obrtel, Praha — T. Ørskov, København — Pereira Filho, Porto Alegre — B. Peyronel, Torino — A. Pilat, Praha — P. Pinetti, Cagliari — A. Pinto Viegas, Campinas — J. Politis, Athènes — G. Pollacci, Pavia — T. Rayss, Jerusalem — I. Reichert, Rehovot — F. Reiss, New York — E. Rivalier, Paris — K. Sakaguchi, Tokyo — R. Sartory, Strasbourg — G. H. Scherr, Chicago Heights — J. Schwarz, Cincinnati — F. J. Seaver, Winter Park, Fla. — G. Ségretain, Paris — R. Singer, Tucumán — A. H. Smith, Ann Arbor, Mich. — F. K. Sparrow, Ann Arbor, Mich. — J. A. Stevenson, Beltsville, Md. — Y. Takahashi, Sendai — M. J. Thirumalachar, Pimpri, Poona — A. Trotter, Vittorio Veneto — N. van Uden, Lisboa — A. C. Ukil, Calcutta — R. Vanbreuseghem, Anvers — C. Vermeil, Tunis — O. Verona, Pisa — G. A. de Vries, Baarn — S. A. Waksman, New Brunswick, N. J. — J. Walker, London — L. E. Wehmeyer, Ann. Arbor, Mich. — J. Westerdijk, Baarn.

MECHANISM OF FUNGAL PATHOGENICITY

A round table discussion, held during the 61st Annual Meeting of the American Society for Microbiology, Chicago, Ill. April 25,1961

UITGEVERIJ Dr. W. JUNK — DEN HAAG — 1961

COMMONWEALTH MYCOLOGICAL INSTITUTE
Mm4 609
30 MAR 1962

MECHANISM OF FUNGAL PATHOGENICITY

A round table discussion, held during the 61st Annual Meeting of the American Society for Microbiology, Chicago, Ill. April 25, 1961

At the initiative of the Chicago Medical Mycological Society, through its president, JOHN W. RIPPON, a round table discussion was organized, during which eight short, invited papers were presented. The meeting was well attended (standing room only!) and at the end of the presentations a lively discussion followed. Under the leadership of the able moderator, ESTHER MEYER, the program had a full success. A condensed report of the events, based on the abstracts of the authors, here follows.

1) ST. MARCUS, G. A. HILL and R. A. KNIGHT reported on the **Induction of Systemic Mycotic Infection in Laboratory Animals**

A previously described (Bact. Proc. p. 88, 1952) standardized and reproducible method for the measurement of mouse virulence (lethality or infection) of systemic mycotic organisms was discussed in the light of subsequent work. The method has yielded interesting information such as the following:

1. Intravenously injected graded doses of yeast phase organisms of strains of *H. capsulatum*, *B. dermatitidis*, *Cr. neoformans*, and *C. albicans* result in mortality related to challenge dose; mice that survive an LD_{50} dose are invariably infected.

2. As long as the organisms are kept at 37° C and subcultured at weekly to monthly intervals, the population will show stable virulence characteristics.

3. Storage of slants at refrigerator temperature may result in depression of virulence.

4. Repeated (15 times) mouse passage may or may not enhance the virulence of different strains of these organisms.

5. Marked variations exist in virulence of strains of these organisms. We have studied particularly 2 strains of *H. capsulatum* and 2 strains of *B. dermatitidis* which are markedly different in virulence (*H. capsulatum*: strain G17M—LD_{50} = ca. 10^6, and of 6651 = 5×10^6; *B. dermatitidis*: strain 410—LD_{50} = 32 to 406 organisms and of strain 380 = $>10^6$).

6. Intraperitoneal or subcutaneous injection of vaccines prepared as formalin killed yeast phase suspensions protects against intravenous or subcutaneous challenge.

7. The degree of resistance engendered by chronic infection with *H. capsulatum* was not observably greater than that induced by immunization.

8. Resistance following infection or immunization does not appear to depend primarily on humoral factors but rather on changes induced in capacity for phagocytic cells to destroy ingested organisms.

9. Mice are readily immunized against infection with polysaccharides derived from *H. capsulatum*.

How real, in terms of human affliction, these results may be cannot be answered. We do know that mouse virulence does not reflect guinea pig virulence. We have found difficulty in inducing progressive histoplasmosis in guinea pigs and failed completely with cynomolgous (*Macacus irus*) monkeys, although others have successfully infected *M. rhesus* and *M. mulatta*. Assessment of significance of results obtained with mouse experiments remains open.

. . .

2) JAN SCHWARZ discussed

The Dog as Index of Endemicity of Mycoses.

EMMONS suggested that the infection rate of dogs in an endemic area of Histoplasmosis could be taken very well as an index of the prevalence of disease. When applied in Cincinnati, which is in the endemic area of histoplasmosis, it was found that in a group of 100 dogs with morphologic methods, 20 were found to have residuals of histoplasmosis. In a subsequent study 132 stray dogs were examined and combining morphologic and cultural techniques, 39 % were found to be positive. Similarly in Tucson, Arizona, which is in the endemic area of coccidioidomycosis, in 194 dogs, 26 % of positivity was obtained. The explanation why dogs which are clinically healthy are carriers of virulent organisms is hard to give.

. . .

3) C. W. EMMONS spoke about

Isolation of Histoplasma Capsulatum from Soil in Washington, D. C.

Reported isolations from soil of *Histoplasma capsulatum* have been generally from specimens collected in rural areas. In some cases of urban human histoplasmosis the patients had an exposure to manure, guano, or top soil imported from rural areas, and a rural source of infection has been generally sought or assumed. Exposure to composted chicken manure has been the most frequent association noted.

Isolation of *H. capsulatum* has been reported from a school yard

and from a large neglected park overgrown with bushes and vines. Its growth in both these areas was associated with the presence of starlings. Results of skin testing have suggested that there may be frequent urban exposure to *H. capsulatum*. It has been suggested that the house bat, *Eptesicus fuscus* may be a factor in permitting growth of the fungus near urban dwellings.

H. capsulatum was isolated, in the investigation reported here, from 10 of 10 specimens of soil collected in a small park adjacent to Pennsylvania Avenue in downtown Washington. This park contains several small sycamore trees which are roosting sites of starlings. The fungus was isolated also from one of five soil specimens collected under oak trees near Massachusetts Avenue and E Street, N. E., in Washington.

The public health aspects of these first isolations of *H. capsulatum* from a busy shopping area of a large city require further investigation. Virulent strains of *H. capsulatum* and *Cryptococcus neoformans* are now known to occur in congested urban areas. [1]).

. . .

4) S. B. SALVIN and R. F. SMITH reported on

Delayed Hypersensitivity and Cryptococcosis.

Sensitization of guinea pigs with minute quantities of highly purified soluble proteins has resulted in delayed hypersensitivity followed in time by Arthus type hypersensitivity and by detectable circulating antibody. From such experiments conclusions were drawn (a) that the specificity of delayed hypersensitivity is directed toward broad, basic portions of the antigen molecule, whereas the specificity of circulating antibody is directed toward smaller, terminal areas of the antigen, and (b) that delayed hypersensitivity may be an intermediate phase in the immune process leading to the formation of circulating antibody. Polysaccharide from *Cryptococcus neoformans* may be considered to act as a hapten, and therefore can combine with antibody in serologic tests but is not active in inducing or detecting delayed hypersensitivity. Protein preparations from *C. neoformans* on the contrary can induce delayed hypersensitivity in guinea pigs and are active for the detection of delayed hypersensitivity in experimentally infected guinea pigs. This preparation did not cross-react in skin reactions in guinea pigs infected with *Histoplasma capsulatum, Coccidioides immitis,* and *Candida albicans*. However, protein preparations obtained from different isolates within the species of *C. neoformans* did cross-react.

. . .

[1]) This report will appear in full in Public Health Reports.

5) M. HUPPERT discussed the

Role of Hypersensitivity in Pathogenesis of Coccidioidomycosis.

Empirical observations indicate that, in human cases of coccidioidomycosis, delayed-type hypersensitivity is associated constantly with resistance to the spread of the infection. More than 99 % of the clinical non-disseminated cases yield a positive skin test, whereas only 30 % of cases with disseminated disease are skin test positive. The incidence in the latter instance can be increased to 60 % positive when the concentration of the skin test material, coccidioidin, is increased tenfold. In the disseminated disease cases, in whom a positive skin test has been maintained, there has been a 75 % survival rate, but this has been reduced to 20 % in those patients with a negative skin test. It appears, therefore, that persistent hypersensitivity parallels resistance to spread of the infection and is indicative of a favorable outcome, but that loss of hypersensitivity parallels progression of the disease and has an unfavorable prognosis.

There is no direct experimental evidence to establish whether this delayed-type hypersensitivity is the cause of resistance to spread of the infection or merely develops independently, but parallel, to such resistance. On the basis of studies which have been reported separately by UHR, LAWRENCE, PAPPENHEIMER, SALVIN, CHASE, and GELL, the author proposed a hypothesis to explain the relationship between hypersensitivity and resistance to spread of infection in coccidioidomycosis. The conclusion reached was that delayed type hypersensitivity functions initially as a host defense mechanism, but that it contains a tissue destructive component which can be dangerous to the host in the presence of excess antigen and serum antibody.

. . .

6) D. B. LOURIA reported on

The Pathogenesis of Experimental Infections due to Cryptococcus and Candida.

Host defenses against Cryptococcal infection are generally considered to be inadequate. Data were presented to show that defense mechanisms in the mouse are in actuality quite good. Immunization with living Cryptococci provided protection against subsequent challenge with ordinarily lethal inocula. Infection with small numbers of *C. neoformans* was associated with progressive increase in fungus census within all tissues during the initial 7 to 14 days after inoculation. Thereafter tissue populations in the majority of animals fell progressively so that tissues were virtually sterile 3 to 6 months after the onset of infection. Mice challenged with lethal inocula at this time demonstrated marked prolongation of

life and increase in survivorship. The studies suggested that defenses against *Cryptococcus* are antibody dependent and maybe expected to be generally adequate for control of the parasite. They also suggest that the localization of infection to the central nervous system in previously healthy humans and the widespread extra-renal involvement observed in persons with malignant disease of the reticulo-endothelial system may be ascribed to the presence of suboptimum amounts of antibody crossing into the central nervous system in the former instance and to the inadequacy of host immune responses in the latter.

In animals infected with *C. albicans* progressive disease is limited to a single organ, the kidney. Serial histologic studies suggested that the renal localization was related to the ability of the fungus to rupture into and grow within the renal tubular lumen, a site in which the fungus appears to be protected from host cellular defense mechanisms. Such renal localization was observed with strains of different virulence. The more virulent strains differed from the less virulent strains in their ability to produce rapid death in the absence of microbial multiplication presumably by production of exotoxins or endotoxins. Furthermore, the more virulent strains show a striking ability to grow out of viable polymorphonuclear leukocytes following phagocytosis. It is suggested that this ability to form mycelia within host cells contributes to toxicity and/or to renal parasitism and represents an important biologic phenomenon in the pathogenesis of candidiasis.

. . .

7) H. F. Hasenclever and W. O. Mitchell spoke about

Antigenic Studies with Candida.

III. Comparative pathogenicity of C. albicans Group A, Group B, and C. stellatoidea.

Antigenic groups have been observed and reported in *Candida albicans*. Further investigation indicated the antigenic similarity of *C. albicans* Group A with *C. tropicalis* and *C. albicans* Group B with *C. stellatoidea*. This investigation was undertaken to determine if any differences in virulence could be detected between strains of *C. albicans* Group A and *C. albicans* Group B strains. *C. stellatoidea* strains were also included in the study. Similar experiments with *C. tropicalis* have been reported.

Twenty-four strains of *C. albicans* Group A, 23 strains of *C. albicans* Group B, and 11 strains of *C. stellatoidea* were studied with respect to mouse virulence. Five *C. albicans* Group B strains and five *C. stellatoidea* strains were tested for lethality in rabbits.

The virulence for mice of both antigenic groups of *C. albicans* was approximately the same. Although *C. stellatoidea* was generally less virulent for mice than *C. albicans*, some strains were more

virulent for mice than the least virulent *C. albicans* strains. All five *C. albicans* Group B strains tested in rabbits demonstrated considerably more virulence for this laboratory animal than any of the four *C. stellatoidea* strains tested. [1]).

. . .

8) F. J. Roth discussed the

Inhibition of Growth of Pathogenic Yeasts by Human Serum.

The serum of normal adults possesses marked capacity to inhibit the *in vitro* growth of *Candida albicans*. In patients with acute blood dyscrasias this inhibitory factor is greatly diminished. Sera from patients with Hodgkin's disease, multiple myeloma, acute leukemia, and erythemic myelosis are especially low in titer. No such reduction is observed in the serum of individuals with cutaneous moniliasis, diabetes, systemic mycoses, and metastatic carcinomas. The growth of all species of *Candida* associated with man are inhibited to some degree by normal serum; *C. albicans* appears to be the most resistant to this growth retarding mechanism. The inhibitory action is not associated with classical antibodies as absorption with diverse forms of homologous and heterologous antigen preparations fail to influence the activity of treated serum. The activity of serum is independent of carbohydrate concentration and is only moderately affected by dialysis. The inhibitory capacity of normal serum is little effected by exposure to 56° C but is largely destroyed by heating at 60° C. for 1—2 hours. The utilization of a variety of nitrogen sources and increased dextrose and vitamin concentrations fail to significantly diminish the inhibitory activity of sera as measured in the assay procedure. Serum from subjects receiving oral therapeutic doses of tetracyclines for 12 days or more failed to show significant decrease in ability to inhibit the growth of *C. albicans*. The interdependent role of trace elements, vitamins, and serum proteins in the total inhibitory mechanism suggests a complex of operative factors in the *in vitro* situation. The role of the inhibitory factors in the pathogenesis of candidiasis is under investigation.

. . .

The invited speakers presented a wealth of excellent material of their own current research. The Chicago Medical Mycological Society deserves great credit for the succesful arrangement of this round table discussion.

Tibor Benedek

[1]) A complete report of this investigation will appear in the Journal of Bacteriology.

The Participants of the Round Table

1) Dr. STANLEY MARCUS, Professor, Department of Bacteriology, University of Utah, College of Medicine, Salt Lake City, Utah.
2) Dr. JAN SCHWARZ, Department of Pathology and Clinical Laboratories of the Jewish Hospital of Cincinnati, Ohio.
3) Dr. CHESTER W. EMMONS, Chief, Medical Mycology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Md.
4) Dr. S. B. SALVIN, Scientist Director of the National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana.
5) Dr. MILTON HUPPERT, Chief, Mycology Research Laboratory, Veterans Administration Hospital, San Fernando, Calif.
6) Dr. DONALD B. LOURIA, Head, Infectious Disease Laboratory, Bellevue Hospital, Second (Cornell) Medical Division, New York, N. Y.
7) Dr. H. F. HASENCLEVER, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Md.
8) Dr. FRANK ROTH, Associate Professor of Dermatology and Microbiology, University of Miami, School of Medicine, Coral Gables, Florida.